the CASP8 competition. This work suggests that physics-based simulations provide an important complement to bioinformatics structure prediction methods.

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3375-Pos Board B422

Accuracy of Ion Channels Homology Models is Significantly Improved by Symmetry-Restrained Molecular Dynamics Simulations

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Ion channels are an important target for drug development, however 3-D structures of biomedically relevant targets are usually unknown. Structural information required for structure-based drug design is often filled by homology models. Making models sufficiently accurate is challenging because few templates are available and these often have substantial structural differences. Most crystallized homo-oligomeric ion channels are highly symmetric, which dramatically decreases conformational space. In molecular dynamics (MD) simulations, channels deviate from the ideal symmetry and accumulate thermal defects. We have tested whether incorporating symmetry restraints in the MD simulations stage improves the accuracy of homology models. Our testing set consisted of three crystal structures of distantly related channels in closed conformation (KcsA, NaK and KirBac3.1), from which six homology models, two for each channel, were built using the remaining two structures as templates. These were embedded in POPC bilayer, solvated, and subjected to unrestrained MD simulations for 8 ns. Two approaches were then used to restore symmetry: 1) symmetry annealing gradually imposed symmetry through soft harmonic restraints during short 1ns simulations. 2) instantaneous symmetrization involved averaging the structures at a given timestep. Both techniques were followed by 8ns unrestrained simulation. This process was repeated three times. Our results show that the symmetry-annealing method improved the accuracy of homology models in 4 out of 6 cases, decreasing the RMSD against the x-ray structure by ~30%. The symmetricized models are also more stable during subsequent unrestrained simulations. The pore of the channel, which is the drug binding region, is improved most, making the modeled structures suitable for drug design. Instantaneous symmetrization produced effects similar to the gradual annealing, but was not as effective in mimicking the target protein's crystal structure and lowered structure stability.

3376-Pos Board B423

The combination of Small-Angle X-ray Scattering fitting and protein structure modeling in Integrative Modeling Platform

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We have shown that Small Angle X-ray Scattering (SAXS) data can be combined with protein structure modeling to determine the quaternary structures of multi-domain proteins and multi-subunit assemblies (Förster et al., J.Mol.-Biol., 2008, 382(4):p. 1089-1106). To maximize the utility of this approach, we further improved the scoring and sampling algorithms, and implemented them in our Integrative Modeling Platform (IMP) software (http://salilab.org/imp). This implementation will facilitate further integration of different kinds of data for determining the structures of proteins and their assemblies.

3377-Pos Board B424

Cryo-EM Guided de novo Protein Fold Elucidation

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Using cryo-electron microscopy (cryoEM) numerous sub-nanometer resolution density maps of large macromolecular assemblies have been reported recently. Although generally no atomic detail is resolved in these density maps, at 7 Å resolution α-helices are observed as density rods. Here we present the development of a computational protein structure prediction algorithm that incorporates the experimental cryoEM data as restraints. The placement of helices is restricted to regions where density rods are observed in the cryoEM density map. The Monte Carlo based protein folding algorithm is further driven by knowledge based energy functions.

The method has been benchmarked with ten highly α-helical proteins of known structure. The chosen proteins range in size from 250 to 350 residues. Starting with knowledge of the true secondary structure for these ten proteins, the method can identify the correct topology within the top scoring 10 models. With more realistic secondary structure prediction information, the correct topology is found within the top scoring 5 models for seven of the ten proteins. The algorithm has been applied to human adenovirus protein IIIa. This protein, for which there is no high resolution structure, is predicted to be highly α-helical. It is resolved in a 6.9Å resolution cryoEM adenovirus structure as a bundle of ~13 α-helical density rods.

3378-Pos Board B425

Membrane Protein Structure Determination by Coupling Sparse Experimental Data with Protein Structure Prediction Techniques

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Membrane protein structure determination by classic experimental methods such as X-ray crystallography and nuclear magnetic resonance (NMR) continues to be extremely challenging, as demonstrated by the extremely low proportion of such structures in the Protein Data Bank. However, more than 50% of pharmaceuticals target membrane proteins, and it is estimated that membrane proteins make up 30-40% of all proteins. Therefore, a novel method was developed for membrane protein structure determination, taking advantage of alternative experimental techniques which are not hindered by the size or environment of membrane proteins. These alternative experimental techniques, such as electron paramagnetic resonance (EPR) and cryo-electron microscopy, provide sparse or low resolution structural data but cannot alone uniquely define a protein's structure. In order to obtain atomic detail models, the method incorporates one or more types of sparse or low resolution experimental data into a protein structure prediction algorithm. The method was benchmarked on a set of membrane proteins with known structure using sparse or low resolution data. This demonstrated the feasibility of obtaining membrane protein models of biologically relevant quality. The method was then applied to the multidrug resistance membrane transporter protein EmrE, for which extensive EPR and electron density data exist, giving a model with a high confidence of being a valid structure for EmrE.

Voltage-gated K Channels-Gating III

3379-Pos Board B426

Voltage-Clamp Fluorimetry Of Kv1.2 Channels Show Two Unique Phases Of Quenching Associated With Channel Activation

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Voltage-clamp fluorimetry can be used to visualize real-time changes in protein structure relative to a locally introduced fluorophore, and thus enhance our understanding of ion channel gating. Since the crystal structure is available for Kv1.2, it seems important to compare the voltage-dependent fluorescence reports from this channel with those already recorded from Shaker potassium channels. In the absence of introduced cysteine residues, we were unable to record voltage-dependent fluorescence signals from wild-type channels incubated with tetramethylrhodamine maleimide (TMRM). However, signals were obtained from an introduced cysteine at A291 in the Kv1.2 S3-S4 linker. Depolarization resulted in two separate components of quenching, which may underlie two different conformational changes in the protein. A slow quenching phase was observed upon depolarization from a holding potential of −120 mV and was essentially complete by −50 mV. The rate of this quenching was not significantly voltage-dependent, with time constants between 35 ms at −70 mV and 29 ms at +80 mV. The voltage-dependence of the slow component suggests that it reports on conformational changes preceding opening. A more rapid quenching component was observed upon depolarizations positive to −40 mV, with time constants from 2-10 ms. It had a similar voltage-dependence to the conductance-voltage relationship for potassium currents through Kv1.2, suggesting that it may report on channel rearrangements associated with opening. Dissociation of channel gating charge movement from pore opening by the incorporation of the ILT triple mutation in the S4 domain abolished the fast phase of fluorescence quenching at potentials up to +80 mV. This suggests that the fast quenching reports on conformational changes associated with channel opening, and that slow fluorescence quenching reflects protein rearrangements occurring earlier in the gating process.

3380-Pos Board B427

Kinetics Of Open- And Closed-state Inactivation Of Kv1.5 At Low pH Or With Ni2+ Ions

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Previous studies have shown that Ni^{2+}_o and H^+_o inhibit Kv1.5 current by enhancing open state inactivation (OSI) and promoting closed state inactivation